

CLAIMS

1. A method for the manufacture of a nucleic acid molecule comprising the steps of

- a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,
- b) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,
- c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,
- d) immobilising the first ligation product to the surface via the modification,
- e) cutting the immobilised ligation product with the first type IIS restriction enzyme thus releasing an elongated oligonucleotide having an overhang,
- f) combining the elongated oligonucleotide with a further at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled, to a surface, whereby the further oligonucleotide comprises a recognition site for a further type IIS restriction enzyme which cuts outside its recognition site and which oligonucleotide comprises a single-stranded overhang, and ligating the elongated second oligonucleotide and the further at least partially double-stranded oligonucleotide via their overhangs forming a further ligation product,
- g) immobilising the further ligation product to a surface via the modification,

- h) cutting the further ligation product with the further type IIS restriction enzyme releasing an elongated oligonucleotide having an overhang, and
- i) optionally, repeating steps f) to h).

2. A method for the manufacture of a nucleic acid molecule comprising the steps of

- a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,
- b) immobilising the first oligonucleotide to the surface via the modification,
- c) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,
- d) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,
- e) cutting the immobilised ligation product with the first type IIS restriction enzyme thus releasing an elongated oligonucleotide having an overhang,
- f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be specifically coupled to a surface, whereby the oligonucleotide contains a recognition site for a further type IIS restriction enzyme and a single-stranded overhang,
- g) immobilising the further at least partially double-stranded oligonucleotide on a surface via its modification,

- h) combining the elongated oligonucleotide with the immobilised further oligonucleotide, and ligating them via their overhangs forming a further ligation product,
- i) cutting the resulting further ligation product with the further type IIS restriction enzyme releasing an elongated oligonucleotide having an overhang, and
- j) optionally, repeating steps f) to i).

3. A method for the manufacture of a nucleic acid molecule comprising the steps of

- a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang,
- b) providing a second at least partially double-stranded oligonucleotide whereby the oligonucleotide comprises a recognition site or a part thereof or a sequence which is complementary thereto, for a second type IIS restriction enzyme which cuts outside its recognition site, and which second oligonucleotide comprises a single-stranded overhang,
- c) ligating the first and the second oligonucleotide via their overhangs generating a first ligation product,
- d) cutting the ligation product with the first type IIS restriction enzyme thus generating an elongated oligonucleotide having an overhang and a shortened first oligonucleotide,
- e) immobilising the shortened first oligonucleotide on a surface via the modification,

- f) providing a further at least partially double-stranded oligonucleotide which has a modification allowing the further oligonucleotide to be coupled to a surface, whereby the further oligonucleotide comprises a recognition site for a further type IIS restriction enzyme which cuts outside its recognition site and which oligonucleotide comprises a single-stranded overhang.
- g) combining the elongated oligonucleotide with the further oligonucleotide and ligating the elongated oligonucleotide and the further oligonucleotide via their overhangs forming a further ligation product,
- h) cutting the further ligation product with the further type IIS restriction enzyme generating an elongated oligonucleotide having an overhang and a shortened further oligonucleotide, and
- i) optionally, repeating steps e) to h).

4. The method according to any of claims 1 to 3, wherein the overhang is a 5'-overhang or a 3'-overhang.

5. The method according to any of claims 1 to 4, wherein the overhang is selected from the group comprising a one nucleotide overhang, a two nucleotides overhang, a three nucleotides overhang, a four nucleotides overhang, a five nucleotides overhang, a six nucleotides overhang and a seven nucleotides overhang.

6. The method according to any of claims 1 to 5, wherein the elongated oligonucleotide is transferred to a new reaction vessel where it is combined with the further oligonucleotide.

7. The method according to any of claims 1 to 6, wherein the at least partially double-stranded oligonucleotide comprises a constant region and a variable region whereby the constant region contains a recognition site for a type IIS restriction enzyme, and the variable region contains a nucleic acid sequence which corresponds to a part of the nucleic acid sequence of the nucleic acid molecule to be manufactured.

8. A method for the synthesis of a nucleic acid molecule comprising the following steps:

- a) Providing a first ligated elongated oligonucleotide by
 - i) providing a first elongated oligonucleotide, whereby the first elongated oligonucleotide is preferably the elongated oligonucleotide according to the method of any of claims 1 to 7;
 - ii) providing a second elongated oligonucleotide, whereby the second elongated oligonucleotide is preferably generated starting from the further ligation product according to the method of any of claims 1 to 7 by cutting the further ligation product by the second type IIS restriction enzyme;
 - iii) ligating the first and the second elongated oligonucleotide, whereby either the first and the second elongated oligonucleotides are ligated in solution and are subsequently immobilized to a surface by means of the modification, or the second elongated oligonucleotide is immobilized to a surface by means of the modification and subsequently the first elongated oligonucleotide is ligated thereto generating in both cases a first ligated elongated oligonucleotide,
- b) providing a second ligated elongated oligonucleotide by
 - i) providing a third elongated oligonucleotide, whereby the third elongated oligonucleotide is the elongated oligonucleotide according to the method of any of claims 1 to 7;
 - iv) providing a fourth elongated oligonucleotide, whereby the fourth elongated oligonucleotide is generated starting from the further ligation product according to the method of any of claims 1 to 7 by cutting the further ligation product by the second type IIS restriction enzyme;
 - v) ligating the third and the fourth elongated oligonucleotide, whereby either the third and the fourth elongated oligonucleotides are ligated in solution and subsequently immobilized to a surface by means of the modification, or the fourth elongated oligonucleotide is immobilized to a surface by means of the modification and subsequently the third elongated oligonucleotide is ligated thereto generating in both cases a second ligated elongated oligonucleotide,
- c) cutting the first ligated elongated oligonucleotide by a type IIS restriction enzyme, whereby the restriction enzyme is the first type IIS restriction enzyme, generating a first cut ligated elongated oligonucleotide;

- d) cutting the second ligated elongated oligonucleotide by a type IIS restriction enzyme, whereby the restriction enzyme is the second type IIS restriction enzyme, generating a second cut ligated elongated oligonucleotide;
- e) combining and ligating the first cut ligated elongated oligonucleotide and the second cut ligated elongated oligonucleotide;
- f) optionally repeating steps a) to e), whereby the ligation product of step e) is used as a first ligated elongated oligonucleotide and/or as a second ligated elongated oligonucleotide.

9. A method for the manufacture of a nucleic acid molecule comprising the steps of

- a) providing a first at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide comprises a recognition site for a first type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide comprises a single-stranded overhang, and whereby the oligonucleotide comprises a part of the nucleic acid molecule to be manufactured,
- b) immobilizing the first oligonucleotide on a surface
- c) cutting the first oligonucleotide with the first type IIS restriction enzyme releasing a double stranded oligonucleotide having a single stranded overhang at each end and being a part of the nucleic acid molecule to be manufactured,
- d) combining the double stranded oligonucleotide of step c) with a second at least partially double-stranded oligonucleotide which has a modification allowing the oligonucleotide to be coupled to a surface, whereby the oligonucleotide contains a recognition site for a second type IIS restriction enzyme which cuts outside its recognition site, and which oligonucleotide further comprises a single-stranded overhang and a part of the nucleic acid molecule to be manufactured, and ligating the double-stranded oligonucleotide of step c) with the second oligonucleotide;

whereby the overhang of the second oligonucleotide is essentially complementary to the overhang of the double stranded oligonucleotide of step c).

10. The method according to claim 9, wherein the overhang generated upon cutting the first oligonucleotide with the first type IIS restriction enzyme is essentially complementary to the overhang of the second at least partially double stranded oligonucleotide.

11. A method for the manufacture of a nucleic acid molecule comprising the following steps:

- a) providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the first and the second type IIS restriction enzymes each generate an overhang, whereby the overhang generated by the first type IIS restriction enzyme has a length which is different from the length of the overhang generated by the second type IIS restriction enzyme;
- b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a third type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a fourth type IIS restriction enzyme and a third oligonucleotide moiety, whereby the third oligonucleotide moiety is a part of the nucleic acid molecule to be manufactured, and whereby the third and the fourth type IIS restriction enzymes each generate an overhang, whereby the overhang generated by the third type IIS restriction enzyme has a length which is different from the length of the overhang generated by the fourth type IIS restriction enzyme;
- c) cutting the first ligation product with the second restriction enzyme generating a first cut ligation product and cutting the second ligation product with the fourth restriction enzyme generating a second cut ligation product;
- d) providing a third at least partially double-stranded oligonucleotide and ligating the third oligonucleotide with the first cut ligation product, whereby the third oligonucleotide comprises an overhang which is complementary to the overhang

of the first cut ligation product generated in step c) and whereby the third oligonucleotide comprises a recognition site for a fifth IIS restriction enzyme;

- e) providing a fourth at least partially double-stranded oligonucleotide and ligating the fourth oligonucleotide to the second cut ligation product, whereby the fourth oligonucleotide comprises an overhang which is complementary to the overhang of the second ligation product generated in step c) and whereby the fourth oligonucleotide comprises a recognition site for a sixth type IIS restriction enzyme;
- f) optionally immobilising the ligation product of step d) and step e) on a surface by means of a modification of the third oligonucleotide and the fourth oligonucleotide;
- g) cutting the immobilised ligation product of step d) with the fifth type IIS restriction enzyme releasing an oligonucleotide;
- h) cutting the immobilised ligation product of step e) with the third type IIS restriction enzyme; and
- i) combining and ligating the oligonucleotide released according to step g) with the immobilised reaction product of step h),

whereby the overhang generated by the first and the third restriction enzyme is complementary to the overhang generated by the fifth and sixth restriction enzyme.

12. The method according to claim 11, wherein the first and the third restriction enzyme are identical and/or the second and the fourth restriction enzyme are identical and/or the fifth and the sixth restriction enzyme are identical.

13. The method according to claim 11 or 12, wherein the first and the third restriction enzyme and the fifth and the sixth restriction enzyme are each a restriction enzyme generating a four nucleotide overhang, preferably at the 5' end.

14. The method according to any of claims 11 to 13, wherein the second and the third restriction enzyme is a restriction enzyme creating an overhang having a length which is selected from the group comprising 1, 2, 3, 4, 5 and 6 nucleotides.

15. The method according to any of claims 11 to 14, wherein the first and the second restriction enzyme is Esp3I or Eco31I and the fifth and the sixth restriction enzyme is Ecp31I or Esp3I.

16. The method according to any of claims 11 to 15, wherein the ligation product of step i) is used as a first ligation product and/or a second ligation product and steps a) to i) are repeated one or several times.

17. The method according to any of claims 11 to 16, wherein the third moiety is arranged between the moieties of the oligonucleotides containing the restriction site for the type IIS restriction enzymes.

18. The method according to any of claims 11 to 17, wherein the first and the second ligation products are provided in separate reaction vessels.

19. A method for the manufacture of a nucleic acid molecule comprising the following steps:

a) Providing a first ligation product, whereby the first ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, and immobilising the first ligation product via a modification to a surface, whereby the modification is incorporated by the second moiety,

b) providing a second ligation product, whereby the second ligation product consists of a first oligonucleotide moiety comprising a recognition site for a first type IIS restriction enzyme, a second oligonucleotide moiety comprising a recognition site for a second type IIS restriction enzyme and a third oligonucleotide moiety, and immobilising the second ligation product via a modification to a surface, whereby the modification is incorporated by the second moiety,

c) cutting the first ligation product with the restriction enzyme the recognition site of which is contained in the first moiety providing a cut immobilised first ligation product,

d) cutting the second ligation product with the restriction enzyme the recognition site of which is contained in the first moiety providing a cut immobilised first ligation product,

e) cutting the cut immobilised first ligation product with the restriction enzyme the recognition site of which is contained in the second oligonucleotide moiety releasing a double-stranded DNA fragment,

f) combining and ligating the double-stranded DNA fragment with the cut immobilised second ligation product.

20. The method according to claim 19, wherein the ligation product of step f) is combined and ligated with an elongated oligonucleotide according to any of the preceding claims, whereby this ligation product is used as a first or a second ligation product in step a) or step b) in the method of claim 19.

21. The method according to claim 19 or 20, wherein the DNA fragment is the nucleic acid molecule or part thereof which is to be manufactured.

22. The method according to any of claims 19 to 21, wherein the third moiety is arranged between the moieties of the oligonucleotides containing the restriction site for the type IIS restriction enzymes.